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<b>(21) International Application Number:</b> PCT/US96/14588 <b>(22) International Filing Date:</b> 12 September 1996 (12.09.96)  <b>(30) Priority Data:</b> 60/003,581 12 September 1995 (12.09.95) US 60/010,509 24 January 1996 (24.01.96) US  <b>(71) Applicants:</b> GENENTECH, INC. [US/US]; 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US). THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612 (US). CHILDREN'S HOSPITAL MEDICAL CENTER OF NORTHERN CALIFORNIA [US/US]; 747 52nd Street, Oakland, CA 94609 (US).  <b>(72) Inventors:</b> MRSNY, Randall, J.; 515 Buena Vista, Redwood City, CA 94061 (US). SHEN, Ben-Quan; 2055 32nd Avenue, San Francisco, CA 94116 (US). WIDDICOMBE, Jonathan, H.; 737 Solana Drive, Lafayette, CA 94549 (US).  <b>(74) Agents:</b> MARSCHANG, Diane, L. et al.; Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> CYSTIC FIBROSIS THERAPY  <b>(57) Abstract</b>  The invention provides methods for treating cystic fibrosis and other pathological conditions in mammals using hepatocyte growth factor ("HGF"). The invention also provides articles of manufacture and kits containing HGF.		

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## CYSTIC FIBROSIS THERAPY

Reference to Government Rights

This invention was made with Government support under Grant No. P50 HL42368 awarded by the Department of Health and Human Services, National Heart, Lung, and Blood Institute. The Government  
5 has certain rights to this invention.

Field of the Invention

The invention relates generally to methods and compositions which can be employed in treating mammals having pulmonary or respiratory disease, including cystic fibrosis. The invention also relates  
10 generally to methods and compositions which can be employed in treating mammals having pathological conditions associated with increased  $\text{Na}^+$  and fluid absorption by cells.

Background of the Invention

Various pulmonary and respiratory diseases, such as pneumonia, bronchitis, and cystic  
15 fibrosis, are often accompanied by viscous secretions in respiratory airways of the patient. Obstruction of the airways by such secretions can cause respiratory distress, and in some cases, can lead to respiratory failure and death.

Investigators have compared the  $\text{Na}^+$  and  $\text{Cl}^-$  permeability of normal epithelia and epithelia in patients suffering from such diseases described above. [See, e.g., Welsh et al., *J. Clin. Invest.*, 80:1523-1526  
20 (1987)]. Measurements of short-circuit current (" $I_{sc}$ ") have shown that normal human airway epithelia are predominantly  $\text{Na}^+$  absorbing under baseline conditions while  $\text{Cl}^-$  secretion is relatively small or absent [Smith et al., *J. Clin. Invest.*, 91:1590-1597 (1993)]. Baseline  $\text{Na}^+$  absorption, however, is elevated approximately two-fold in cystic fibrosis ("CF") patients [Boucher et al., *J. Clin. Invest.*, 78:1245-1252 (1986)].

Recently, a cAMP-dependent  $\text{Cl}^-$  channel has been identified to be defective in CF patients.  
25 This channel, referred to as CF transmembrane regulator ("CFTR"), has been suggested to play an important role in normal  $\text{Na}^+$  transport [Stutts et al., *Science*, 269:847-850 (1995)]. Despite the presence of several  $\text{Cl}^-$  channels being present in the apical membrane of airway epithelial cells, it is believed that the CFTR provides the major  $\text{Cl}^-$  conductance pathway in these cells and that this channel is close to maximally activated under baseline conditions [Shen et al., *Am. J. Phys.*, 269:L561-L566 (1995)]. In the absence of significant  $\text{Cl}^-$  efflux,  
30 the increased basal  $\text{Na}^+$  influx (and associated fluid absorption) observed in CF airway epithelia has been suggested to contribute to the formation of the abnormally hydrated, viscous mucous secretions which occlude the airways in CF disease [Jiang et al., *Science*, 262:424-427 (1993)].

Certain pharmacological approaches to rectify the abnormal fluid absorption in CF patients have been described. For instance, some investigators have described stimulating epithelial  $\text{Cl}^-$  secretion using  
35 agents such as uracil 5' triphosphate ("UTP") to raise intracellular calcium ion levels and attenuate  $\text{Ca}^{2+}$  activated  $\text{Cl}^-$  channels [Knowles et al., *N. Engl. J. Med.*, 325:533-538 (1991)]. Another approach has been to use aerosolized amiloride to block  $\text{Na}^+$  absorption [Boucher et al., *supra*]. The present invention is directed to the use of hepatocyte growth factor ("HGF") to modulate fluid absorption by modulating the expression of

Na<sup>+</sup> channels. As described below, the present inventors have surprisingly discovered that HGF produces an effective and prolonged block of amiloride-sensitive Na<sup>+</sup> absorption.

It is recognized in the art that HGF functions as a growth factor for particular tissues and cell types. HGF was identified initially as a mitogen for hepatocytes [Michalopoulos et al., Cancer Res., 44:4414-4419 (1984); Russel et al., J. Cell. Physiol., 119:183-192 (1984); Nakamura et al., Biochem. Biophys. Res. Comm., 122:1450-1459 (1984)]. Nakamura et al., supra, reported the purification of HGF from the serum of partially hepatectomized rats. Subsequently, HGF was purified from rat platelets, and its subunit structure was determined [Nakamura et al., Proc. Natl. Acad. Sci. USA, 83:6489-6493 (1986); Nakamura et al., FEBS Letters, 224:311-316 (1987)]. The purification of human HGF ("huHGF") from human plasma was first described by Gohda et al., J. Clin. Invest., 81:414-419 (1988).

Both rat HGF and huHGF have been molecularly cloned, including the cloning and sequencing of a naturally occurring variant lacking 5 amino acids designated "delta5 HGF" [Miyazawa et al., Biochem. Biophys. Res. Comm., 163:967-973 (1989); Nakamura et al., Nature, 342:440-443 (1989); Seki et al., Biochem. Biophys. Res. Commun., 172:321-327 (1990); Tashiro et al., Proc. Natl. Acad. Sci. USA, 87:3200-3204 (1990); Okajima et al., Eur. J. Biochem., 193:375-381 (1990)].

The mature form of huHGF, corresponding to the major form purified from human serum, is a disulfide linked heterodimer derived by proteolytic cleavage of the human pro-hormone between amino acids R494 and V495. This cleavage process generates a molecule composed of an  $\alpha$ -subunit of 440 amino acids ( $M_r$  69 kDa) and a  $\beta$ -subunit of 234 amino acids ( $M_r$  34 kDa). The nucleotide sequence of the huHGF cDNA reveals that both the  $\alpha$ - and the  $\beta$ -chains are contained in a single open reading frame coding for a pre-pro precursor protein. In the predicted primary structure of mature huHGF, an interchain S-S bridge is formed between Cys 487 of the  $\alpha$ -chain and Cys 604 in the  $\beta$ -chain [see Nakamura et al., Nature, supra]. The N-terminus of the  $\alpha$ -chain is preceded by 54 amino acids, starting with a methionine group. This segment includes a characteristic hydrophobic leader (signal) sequence of 31 residues and the prosequence. The  $\alpha$ -chain starts at amino acid (aa) 55, and contains four kringle domains. The kringle 1 domain extends from about aa 128 to about aa 206, the kringle 2 domain is between about aa 211 and about aa 288, the kringle 3 domain is defined as extending from about aa 303 to about aa 383, and the kringle 4 domain extends from about aa 391 to about aa 464 of the  $\alpha$ -chain.

The definition of the various kringle domains is based on their homology with kringle-like domains of other proteins (such as prothrombin and plasminogen), therefore, the above limits are only approximate. To date, the function of these kringles has not been determined. The  $\beta$ -chain of huHGF shows high homology to the catalytic domain of serine proteases (38% homology to the plasminogen serine protease domain). However, two of the three residues which form the catalytic triad of serine proteases are not conserved in huHGF. Therefore, despite its serine protease-like domain, huHGF appears to have no proteolytic activity, and the precise role of the  $\beta$ -chain remains unknown. HGF contains four putative glycosylation sites, which are located at positions 294 and 402 of the  $\alpha$ -chain and at positions 566 and 653 of the  $\beta$ -chain.

In a portion of cDNA isolated from human leukocytes, in-frame deletion of 15 base pairs was observed. Transient expression of the cDNA sequence in COS-1 cells revealed that the encoded HGF molecule (delta5 HGF) lacking 5 amino acids in the kringle 1 domain was fully functional [Seki et al., supra].

A naturally occurring huHGF variant has been identified which corresponds to an alternative spliced form of the huHGF transcript containing the coding sequences for the N-terminal finger and first two kringle domains of mature huHGF [Chan et al., Science, 254:1382-1385 (1991); Miyazawa et al., Eur. J. Biochem., 197:15-22 (1991)]. This variant, designated HGF/NK2, has been proposed to be a competitive antagonist of mature huHGF.

Comparisons of the amino acid sequence of rat HGF with that of huHGF have revealed that the two sequences are highly conserved and have the same characteristic structural features. The length of the four kringle domains in rat HGF is exactly the same as in huHGF. Furthermore, the cysteine residues are located in exactly the same positions, an indication of similar three-dimensional structures [Okajima et al., supra; Tashiro et al., supra].

HGF and HGF variants are described further in U.S. Patent Nos. 5,227,158; 5,316,921; and 5,328,837.

The HGF receptor has been identified as the product of the c-Met proto-oncogene [Bottaro et al., Science, 251:802-804 (1991); Naldini et al., Oncogene, 6:501-504 (1991); WO 92/13097 published August 6, 1992; WO 93/15754 published August 19, 1993]. The receptor is usually referred to as "c-Met" or "p190<sup>MET</sup>" and typically comprises, in its native form, a 190-kDa heterodimeric (a disulfide-linked 50-kDa  $\alpha$ -chain and a 145-kDa  $\beta$ -chain) membrane-spanning tyrosine kinase protein [Park et al., Proc. Natl. Acad. Sci. USA, 84:6379-6383 (1987)]. Several truncated forms of the c-Met receptor have also been described [WO 92/20792; Prat et al., Mol. Cell. Biol., 11:5954-5962 (1991)].

The binding activity of HGF to its receptor is believed to be conveyed by a functional domain located in the N-terminal portion of the HGF molecule, including the first two kringles [Matsumoto et al., Biochem. Biophys. Res. Commun., 181:691-699 (1991); Hartmann et al., Proc. Natl. Acad. Sci., 89:11574-11578 (1992); Lokker et al., EMBO J., 11:2503-2510 (1992); Lokker and Godowski, J. Biol. Chem., 268:17145-17150 (1993)]. The c-Met protein becomes phosphorylated on tyrosine residues of the 145-kDa  $\beta$ -subunit upon HGF binding.

Various biological activities have been described for HGF and its receptor [see, generally, Chan et al., Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the C-Met Receptor, Goldberg and Rosen, eds., Birkhauser Verlag-Basel (1993), pp. 67-79]. It has been observed that levels of HGF increase in the plasma of patients with hepatic failure [Gohda et al., supra] and in the plasma [Lindroos et al., Hepatology, 13:743-750 (1991)] or serum [Asami et al., J. Biochem., 109:8-13 (1991)] of animals with experimentally induced liver damage. The kinetics of this response are usually rapid, and precedes the first round of DNA synthesis during liver regeneration. HGF has also been shown to be a mitogen for certain cell types, including melanocytes, renal tubular cells, keratinocytes, certain endothelial cells and cells of epithelial origin [Matsumoto et al., Biochem. Biophys. Res. Commun., 176:45-51 (1991); Igawa et al., Biochem. Biophys. Res. Commun., 174:831-838 (1991); Han et al., Biochem., 30:9768-9780 (1991); Rubin et al., Proc. Natl. Acad. Sci. USA, 88:415-419 (1991)]. Both HGF and the c-Met protooncogene have been postulated to play a role in microglial reactions to CNS injuries [DiRenzo et al., Oncogene, 8:219-222 (1993)].

HGF can also act as a "scatter factor", an activity that promotes the dissociation of epithelial and vascular endothelial cells *in vitro* [Stoker et al., Nature, 327:239-242 (1987); Weidner et al., J. Cell Biol.

111:2097-2108 (1990); Naldini et al., EMBO J. 10:2867-2878 (1991); Giordano et al., Proc. Natl. Acad. Sci. USA, 90:649-653 (1993)]. Moreover, HGF has recently been described as an epithelial morphogen [Montesano et al., Cell, 67:901-908 (1991)]. Therefore, HGF has been postulated to be important in tumor invasion [Comoglio, Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the C-Met Receptor, Goldberg and Rosen, eds., Birkhauser Verlag-Basel (1993), pp. 131-165].

#### Summary of the Invention

The invention provides methods for treating cystic fibrosis comprising administering to a mammal diagnosed as having cystic fibrosis an effective amount of HGF. The HGF alone may be administered to the mammal, or alternatively, may be administered to the mammal in combination with other therapies and/or pharmacologic agents, such as described herein.

The invention also provides methods for reducing sodium ion absorption in mammalian cells, comprising exposing said cells to an effective amount of HGF.

The invention further provides articles of manufacture and kits which contain HGF.

Although not being bound by any particular theory, it is presently believed that the HGF can be used to substantially diminish abnormally high amiloride-sensitive  $\text{Na}^+$  and fluid absorption by cells, thereby treating pathologic conditions associated with diseases such as cystic fibrosis. The HGF may also be used to re-establish the epithelia lining damaged passageways by proliferation of epithelial cells.

#### Brief Description of the Figures

Figure 1 includes a bar diagram showing the effect of rhuHGF on proliferation of normal human tracheal cells *in vitro*.

Figure 2A includes a bar diagram comparing the response of normal human tracheal cells to challenge with amiloride, isoproterenol, histamine, methacholine and bradykinin following exposure to 100 ng/ml rhuHGF *in vitro*.

Figure 2B is a graph illustrating the effect of rhuHGF (100 ng/ml) over the course of 72 hours on  $I_{eq}$  of JME/CF 15 cells *in vitro*.

Figure 2C is a graph illustrating the effect of rhuHGF (100 ng/ml) over the course of 72 hours on the transepithelial electrical resistance ( $R_{te}$ ) of JME/CF 15 cell monolayers *in vitro*.

Figure 2D is a graph illustrating the effect of varying doses of rhuHGF (0-500 ng/ml) on  $I_{eq}$  of JME/CF 15 cells *in vitro*.

Figure 3 shows a Western blot illustrating increased c-Met associated phosphotyrosine content of JME/CF 15 cells following exposure to 100 ng/ml rhuHGF.

Figure 4 shows a Western blot illustrating a reduction of the  $\alpha$ -subunit of the amiloride-sensitive  $\text{Na}^+$  channel protein content of JME/CF 15 cells following exposure to 100 ng/ml rhuHGF.

Figure 5 is a graph illustrating the effect of 100 ng/ml rhuHGF over the course of 36 hours on  $I_{eq}$  of primary bronchial epithelial cells obtained from a human cystic fibrosis patient and cultured *in vitro*.

Figure 6 is a graph illustrating the time course effect of 100 ng/ml rhuHGF on the transepithelial electrical resistance ( $R_{te}$ ) of primary bronchial epithelial cells obtained from a human cystic fibrosis patient and cultured *in vitro*.

Figure 7 is a graph comparing the  $I_{sc}$  responses of primary bronchial epithelial cells (obtained from a human cystic fibrosis patient and cultured *in vitro*) to challenge with amiloride, isoproterenol, histamine, and bradykinin following 48 hours exposure to 100 ng/ml rhuHGF.

### Detailed Description of the Invention

#### I. Definitions

As used herein, the terms "hepatocyte growth factor" and "HGF" refer to a growth factor typically having a structure with six domains (finger, Kringle 1, Kringle 2, Kringle 3, Kringle 4 and serine protease domains). Fragments of HGF constitute HGF with fewer domains and variants of HGF may have some of the domains of HGF repeated; both are included if they still retain their respective ability to bind an HGF receptor. The terms "hepatocyte growth factor" and "HGF" include hepatocyte growth factor from humans ("huHGF") and any non-human mammalian species, and in particular rat HGF. The terms as used herein include mature, pre, pre-pro, and pro forms, purified from a natural source, chemically synthesized or recombinantly produced. Human HGF is encoded by the cDNA sequence published by Miyazawa et al., 1989, *supra*, or Nakamura et al., 1989, *supra*. The sequences reported by Miyazawa et al. and Nakamura et al. differ in 14 amino acids. The reason for the differences is not entirely clear; polymorphism or cloning artifacts are among the possibilities. Both sequences are specifically encompassed by the foregoing terms. It will be understood that natural allelic variations exist and can occur among individuals, as demonstrated by one or more amino acid differences in the amino acid sequence of each individual. The HGF of the invention preferably has at least about 80% sequence identity, more preferably at least about 90% sequence identity, and even more preferably, at least about 95% sequence identity with a native mammalian HGF. The terms "hepatocyte growth factor" and "HGF" specifically include the delta5 huHGF as disclosed by Seki et al., *supra*.

The terms "HGF receptor" and "c-Met" when used herein refer to a cellular receptor for HGF, which typically includes an extracellular domain, a transmembrane domain and an intracellular domain, as well as variants and fragments thereof which retain the ability to bind HGF. The terms "HGF receptor" and "c-Met" include the polypeptide molecule that comprises the full-length, native amino acid sequence encoded by the gene variously known as p190<sup>MET</sup>. The present definition specifically encompasses soluble forms of HGF receptor, and HGF receptor from natural sources, synthetically produced *in vitro* or obtained by genetic manipulation including methods of recombinant DNA technology. The HGF receptor variants or fragments preferably share at least about 65% sequence homology, and more preferably at least about 75% sequence homology with any domain of the human c-Met amino acid sequence published in Rodrigues et al., *Mol. Cell. Biol.*, 11:2962-2970 (1991); Park et al., *Proc. Natl. Acad. Sci.*, 84:6379-6383 (1987); or Ponzetto et al., *Oncogene*, 6:553-559 (1991).

The term "cystic fibrosis" when used herein refers to or describes the physiological condition in mammals typically characterized by viscous or mucus secretions that tend to obstruct or occlude various

internal passageways in a mammal, including but not limited to, pancreatic ducts, bile ducts, and respiratory airways.

The terms "treating," "treatment," and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventative therapy.

5           The term "mammal" as used herein refers to any animal classified as a mammal, including humans, cows, horses, dogs and cats. In a preferred embodiment of the invention, the mammal is a human.

## II. Compositions and Methods of the Invention

10           The present invention provides methods for treating cystic fibrosis using hepatocyte growth factor, referred to hereinafter as "HGF". It is also contemplated that HGF will be particularly useful for the treatment of mammals having a pathological condition which is accompanied by abnormal, viscous or inspissated mucus secretions and/or resulting from increased  $\text{Na}^+$  or fluid absorption by cells. Examples of such conditions include acute or chronic bronchopulmonary disease (infectious pneumonia, bronchitis or tracheobronchitis, bronchiectasis, asthma, TB or fungal infections), atelectasis due to tracheal or bronchial  
15           impaction, complications of tracheostomy, and Liddle's Syndrome.

          The HGF useful in the practice of the present invention can be prepared in a number of ways. For instance, the HGF can be prepared using an isolated or purified form of HGF. Methods of isolating and purifying HGF from natural sources are known in the art. Such isolation and purification methods can be employed for obtaining HGF from serum or plasma. Alternatively, HGF can be chemically synthesized and  
20           prepared using recombinant DNA techniques known in the art and described in further detail in Example 1 below.

          The HGF may be from human or any non-human species. For instance, a mammal may have administered HGF from a different mammalian species (e.g., rats can be treated with human HGF). Preferably, however, the mammal is treated with homologous HGF (e.g., humans are treated with human HGF) to avoid  
25           potential immune reactions to the HGF.

          In a preferred embodiment of the invention, methods for treating cystic fibrosis are provided. In the methods, HGF is administered to a mammal diagnosed as having cystic fibrosis. It is of course contemplated that the methods of the invention can be employed in combination with still other therapeutic techniques such as percussion physical therapy, enzyme replacement therapy (such as pancreatic enzymes), and  
30           transplant therapy.

          The HGF is preferably administered to the mammal in a pharmaceutically-acceptable carrier. Suitable carriers and their formulations are described in Remington's Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Co., edited by Oslo et al. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable  
35           carrier include liquids such as saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. The formulation may also comprise a lyophilized powder. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be



more preferable depending upon, for instance, the route of administration and concentration of HGF being administered.

5 The HGF can be administered to the mammal by injection (e.g. intravenous, intraperitoneal, subcutaneous, intramuscular), or by other methods such as infusion that ensure its delivery to the bloodstream in an effective form. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers may also be useful for administration. Liquid formulations may be directly nebulized and lyophilized power nebulized after reconstitution. Alternatively, HGF may be aerosolized using a metered dose inhaler, or inhaled as a lyophilized and milled powder. In addition, the liquid formulation of HGF may be directly instilled in the nasotracheal or endotracheal tubes in intubated patients.

10 Effective dosages and schedules for administering the HGF may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of HGF that must be administered will vary depending on, for example, the mammal which will receive the HGF, the route of administration, the particular type of HGF used and other drugs being administered to the mammal. A typical daily dosage of the HGF used alone might range from about 1  $\mu\text{g/kg}$  to up to 100  
15 mg/kg of body weight or more per day, depending on the factors mentioned above.

HGF may also be administered along with other pharmacologic agents used to treat the conditions listed above, such as UTP, amiloride, DNase, antibiotics, bronchodilators, anti-inflammatory agents, and mucolytics (e.g. n-acetyl-cysteine). It may also be useful to administer HGF along with other therapeutic human proteins such as protease inhibitors, gamma-interferon, enkephalinase, lung surfactant, and colony  
20 stimulating factors. The HGF may be administered sequentially or concurrently with the one or more other pharmacologic agents. The amounts of HGF and pharmacologic agent depend, for example, on what type of drugs are used, the disease being treated, and the scheduling and routes of administration.

Following administration of HGF to the mammal, the mammal's physiological condition can be monitored in various ways well known to the skilled practitioner.

25 In another embodiment of the invention, there are provided articles of manufacture and kits containing materials useful for treating the pathological conditions described herein, including cystic fibrosis. The article of manufacture comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition having an active agent which is effective for treating, for example, cystic  
30 fibrosis. The active agent in the composition is HGF. The label on the container indicates that the composition is used for treating, for example, cystic fibrosis, and may also indicate directions for *in vivo* use, such as those described above.

The kit of the invention comprises the container described above and a second container comprising a pharmaceutically-acceptable carrier or buffer. It may further include other materials desirable  
35 from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All reference citations herein are incorporated by reference.

## Examples

## Example 1

Recombinant human HGF ("rhuHGF") was produced in CHO cells using a procedure modified from Naka et al., J. Biol. Chem., 267:20114-20119 (1992). rhuHGF-transfected cells were grown in a 400 L bioreactor in medium containing 2% fetal bovine serum for 8 days. Culture supernatant containing rhuHGF was concentrated and clarified, then conditioned by the addition of solid NaCl to 0.3 M. rhuHGF was then purified in a single step using cation exchange chromatography. Conditioned, concentrated culture supernatant was loaded onto a column of S-Sepharose™ Fast Flow equilibrated in 20 mM Tris, pH 7.5, 0.3 M NaCl. After washing out unbound protein, rhuHGF was eluted in a linear gradient from 20 mM Tris, pH 7.5, 0.3 M NaCl to 20 mM Tris, pH 7.5, 1.2 M NaCl. rhuHGF-containing fractions were pooled based on SDS-PAGE analysis. The S-Sepharose™ Fast Flow pool was concentrated and exchanged into 20 mM Tris, pH 7.5, 0.5 M NaCl by gel filtration on Sephadex G25 to a final concentration of about 3-5 mg/ml. A rhuHGF stock solution was then prepared by diluting the rhuHGF in assay buffer (0.5% bovine serum albumin, 0.05% Tween-20, 0.01% Thimersol in PBS) to a concentration of 10 µg/ml.

Proliferation of primary isolates of autopsied, normal human tracheal epithelial cells (obtained from University of California, San Francisco Medical Center) was measured by monitoring the growth rate of the cells plated at low density (approximately 2000 cells/cm<sup>2</sup>) on transparent, permeable filters. rhuHGF (at concentrations of greater than 50 ng/ml) markedly increased the cell number and scattering activity after 2-3 days when added at the time of seeding the cells. (See Figure 1). A correlation between rhuHGF concentration and augmented growth rate was also observed.

Differentiation of the cultured tracheal cells was also measured by I<sub>sc</sub> current. The tracheal cells were placed onto Nucleopore cell culture inserts (0.45 micron pore size, 1 cm<sup>2</sup> surface area) coated with human placental collagen, and cultured in 2% USG medium as described in Shen et al., Am. J. Phys., 266: L493-L501 (1994). The cells were treated with 50 ng/ml - 1 µg/ml rhuHGF basolaterally 8-14 days after plating, a point when the cell monolayers had transepithelial resistance (R<sub>te</sub>) > 50 ohm/cm<sup>2</sup> as determined using a "chopstick" voltmeter (Millicell®-ERS, Millipore, Bedford, MA). After 24-48 hours following addition of the rhuHGF, ion transport activities were measured in Ussing chambers using standard methods [Ussing et al., Acta Phys. Scandinav., 23:110-127 (1951); Widdicombe, Methods in Bronchial Mucology, Braga et al., eds., pp. 335-345 (1988)].

Confluent cell sheets were cut out from the filter inserts, and mounted between Lucite half chambers. Transepithelial potential difference was clamped to zero by an external current passing circuit, and the resulting I<sub>sc</sub> was recorded continuously on a chart recorder. R<sub>te</sub> was determined from the current pulses needed to clamp the voltage to 0.5 or 1.0 mV for 20 msec every 20 sec. Warm, oxygenated Krebs-Henseleit solution was circulated across both faces of the filters by gas-lift oxygenators. The cells were then challenged with apical additions of 10 µM isoproterenol ("Iso"), 10 µM histamine ("His"), 10 µM methacholine ("Mch") or 1 µM bradykinin ("BK").

The results are shown in Figure 2A for control cells (receiving no rhuHGF treatment) and for tracheal cells treated with 100 ng/ml rhuHGF. The measured baseline I<sub>sc</sub> is reported as "Isc-bas".

Treatment with rhuHGF enhanced differentiation of the tracheal cells as reflected by the increase in  $I_{sc}$ , which reading was indicative of an increased responsiveness to challenge with  $Cl^-$  secretagogues.

### Example 2

5 JME/CF 15 cells were cultured, and  $R_{te}$  and  $I_{sc}$  were measured as described in Example 1. JME/CF 15 cells (obtained from D. Jefferson, New England Medical Center Hospitals, Inc., Boston, MA) is a cell line derived by SV40-transformation of primary cultures of nasal epithelium from a CF patient. The cell line demonstrates a CF phenotype characterized by a relatively high level of amiloride-sensitive  $Na^+$  absorption and lack of cAMP-activated  $Cl^-$  secretion *in vitro*.

10 The results are shown in Table 1. Changes in short-circuit currents ( $\Delta I_{sc}$ ) following apical addition of  $10^{-5}$  M amiloride (AM) and basolateral challenge with  $10^{-5}$  M isoproterenol (ISO) are given as means  $\pm$  SE (n=6). Tests of statistical significance between means were performed with paired or unpaired Student's t-test.  $P < 0.05$  was regarded as significant.

15 Treatment of the cells with rhuHGF did not significantly affect monolayer resistance, while the baseline  $I_{sc}$  was reduced, as shown in Table 1 below. The rhuHGF-treated cells showed marked decreases in baseline  $I_{sc}$  and sensitivity to amiloride when compared on the same day with control cells. No effect on  $Cl^-$  channel activity was observed, as evidenced by a lack of response to the challenges with isoproterenol, histamine, methacholine or bradykinin. Only a small effect was observed following ouabain addition suggesting that inhibition of  $I_{sc}$  by amiloride was complete, and that amiloride-sensitive sodium absorption accounted for essentially all the  $I_{sc}$ . The effects of amiloride on  $I_{sc}$  were not affected by pretreatment with  $10^{-4}$  M bumetanide for 15 min. in the basolateral medium (data not shown), indicating that differences in sensitivity to amiloride between treated and untreated cells were not due to induction of  $Cl^-$  secretion.

Table 1

25

PARAMETER	CONTROL	100 ng/ml rhuHGF
$R_{te}$	$120 \pm 14 \Omega/cm^2$	$106 \pm 11 \Omega/cm^2$
Baseline $I_{sc}$	$25.3 \pm 1.6 \mu A/cm^2$	$2.1 \pm 0.8 \mu A/cm^2^*$
$\Delta I_{sc}(AM)$	$27.2 \pm 2.0 \mu A/cm^2$	$4.5 \pm 0.8 \mu A/cm^2^*$
$\Delta I_{sc}(ISO)$	$0 \pm 0 \mu A/cm^2$	$0 \pm 0 \mu A/cm^2$

30

\* = significantly different from control value

For comparison, the baseline  $I_{sc}$  values for CF and non-CF tracheal epithelium are approximately 30 and 10  $\mu A/cm^2$ , respectively.

35 In a separate study, JME/CF 15 cell monolayers were cultured with 100 ng/ml rhuHGF (added basolaterally) for up to 72 hours. As shown in Figure 2B, addition of rhuHGF to the basolateral medium decreased  $I_{eq}$  with a  $t_{1/2}$  of approximately 18 hours, and a maximal inhibition of approximately 90%.

The values presented in Figure 2B are means  $\pm$  SE,  $n = 4$ . After 48 hours, 2 filters were continued to be exposed to the rhuHGF, while 2 filters were washed free of the rhuHGF. Washout of rhuHGF at 48 hours resulted in a partial recovery of  $I_{eq}$  24 hours later. (See Figure 2B).  $R_{te}$  values were not affected by rhuHGF treatment during this time course. (Figure 2C).

5 In a dose response experiment over this same time frame, confluent JME/CF 15 monolayers were cultured for 48 hours with varying doses of rhuHGF ranging from 0 to 500 ng/ml (added basolaterally). Baseline short circuit current measurements (mean  $\pm$  SEM,  $n = 4$ ) were compared using a paired student's t-test with  $p < 0.05$  being regarded as significant. As shown in Figure 2D, 100 ng/ml rhuHGF appeared to result in near maximal response, although significant responses were observed at lower rhuHGF levels. ELISA  
10 evaluation [performed as described in Roos et al., *Am. J. Physiol.*, 268:G380-G386 (1995)] of spent media from the cell monolayers demonstrated no detectable levels of rhuHGF were released from these cells and that approximately half of the rhuHGF spiked into media could be recovered after 48 hours of exposure (data not shown).

It is believed that the maximal effect on amiloride-sensitive  $Na^+$  absorption will occur for  
15 approximately 48 hours with continuous rhuHGF exposure. It is believed that recovery of attenuated amiloride-sensitive  $Na^+$  absorption will occur after approximately 12 hours following removal of rhuHGF.

### Example 3

In an assay to determine the effect of rhuHGF on c-Met receptor phosphotyrosine content,  
20 JME/CF 15 cell monolayers were treated with 100 ng/ml rhuHGF (applied basolaterally or apically), lysed and evaluated for a c-Met associated phosphotyrosine content. The JME/CF 15 cell monolayers were lysed in 150 mM NaCl, 50 mM Tris-Cl, pH 8.0, 1% triton X-100, 0.1% SDS, 1% sodium deoxycholate and 1 mM phenylmethylsulfonyl fluoride and subjected to 8% SDS-PAGE. c-Met associated phosphotyrosine content was determined by immunoprecipitating with anti-Met COOH antibodies (Santa Cruz Biotechnology, Santa  
25 Cruz, CA) and blotting with anti-phosphotyrosine antibodies as described in U.S. Patent 5,316,921. Control samples were treated identically but not exposed to rhuHGF.

Figure 3 shows a Western blot of the following samples: molecular weight markers (Lane 1); an antibody control (Lane 2); a JME/CF 15 cell control (Lane 3); a JME/CF 15 cell sample treated basolaterally with rhuHGF for 7 days (Lane 4); a JME/CF 15 cell sample treated basolaterally with rhuHGF  
30 for 15 minutes (Lane 5); a JME/CF 15 cell control (Lane 6); a JME/CF 15 cell sample treated apically with rhuHGF for 15 minutes (Lane 7). It was found that the c-Met receptor is down-regulated following activation by HGF. Further, basolateral exposure of the cells to rhuHGF produced an increase in c-Met receptor phosphotyrosine content. Phosphorylation and down-regulation of c-Met preceded the decreases observed in ENaC expression and function.

35 Previous reports have suggested that the c-Met receptor is expressed only on the basolateral domain of polarized cells [Crepaldi et al., *J. Cell. Biol.*, 125:313-320 (1994)]. No rhuHGF-induced actions were observed following presentation to only the apical surface of the cells and there is a rapid increase in phosphotyrosine of c-Met and a subsequent down-regulation of c-Met following the rhuHGF addition. These results are consistent with a receptor-mediated process.

Sodium channel protein level was also measured by Western blotting with a polyclonal antibody raised against the amiloride-sensitive epithelial  $\text{Na}^+$  channel complex (ENaC) isolated from bovine kidney papillae [Sorscher et al., *Am. J. Physiol.*, 255:C835-C843 (1988); Fuller et al., *Am. J. Physiol.*, 269:C641-C654 (1995)]. Following 48 hour exposure to 100 ng/ml rhuHGF, JME/CF 15 cell monolayers were lysed in 150 mM NaCl, 50 mM Tris-Cl, pH 8.0, 1% triton X-100, 0.1% SDS, 1% sodium deoxycholate and 1 mM phenylmethylsulfonyl fluoride and subjected to 8% SDS-PAGE. Separated proteins were transferred onto a PVDF membrane, blocked with 5% non-fat dry milk, and incubated with 1.0  $\mu\text{g/ml}$  rabbit polyclonal anti-amiloride sensitive  $\text{Na}^+$  channel (ENaC) antiserum overnight. A goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody was then added and an enhanced chemiluminescence kit (Amersham, Arlington Heights, IL) was used to detect the presence of ENaC. Antibody controls were performed the same except that no anti-ENaC antiserum was used.

Figure 4 shows a Western blot of the following samples: rhuHGF-treated cells, no antibody (Lane 1); control cells, no antibody (Lane 2); rhuHGF-treated cells with antibody (Lane 3); control cells with antibody (Lane 4). As shown in Figure 4, levels of a specific 95-97 kDa protein in rhuHGF-treated cells was much lower than in control cells. The molecular weight of this protein is similar to that of the  $\alpha$ -subunit of ENaC (approximately 95 kDa). Thus, it is believed that the rhuHGF may decrease the amiloride-sensitive sodium absorption in part (at least) by down regulating the expression of  $\alpha$ -ENaC.

#### EXAMPLE 4

Primary bronchial epithelial cells from a human CF patient (designated R117H/deltaF508 genotype) were obtained by lung resection and cultured as described in Example 1. The cells were treated with 100 ng/ml rhuHGF (see Example 1). Before and after addition of the rhuHGF, the transepithelial resistance ( $R_{te}$ ) and potential difference (PD) were monitored with the Millicell $\Omega$ -ERS meter, as described in Example 1.

Treatment of the cells with the rhuHGF led to a decline in equivalent short circuit current ( $I_{eq} = \text{PD}/R$ ) with a  $t_{1/2}$  of approximately 10 hours, reaching values close to zero after approximately 36 hours. (Figure 5). In contrast, matched time controls (receiving buffer alone) showed no changes of  $I_{eq}$  over the same time period. (Figure 5) The values presented in time controls in Figure 5 (represented by closed circles) are an average from two different tissues and values presented for rhuHGF-treated monolayers (open circles) are means  $\pm$  SE,  $n=4$ . Transepithelial electrical resistance ( $R_{te}$ ) was not affected by the rhuHGF treatment. (see Figure 6).

After 48 hours of culturing with rhuHGF, single control and HGF treated cells were tested in Ussing chambers, as described in Example 1. Transepithelial potential difference was clamped to zero by an external current passing circuit, and the resulting  $I_{sc}$  was recorded continuously on a chart recorder. As shown in Figure 7, baseline  $I_{sc}$  in the rhuHGF treated cells was lower than that of control ( $14 \mu\text{A}/\text{cm}^2$  vs.  $26 \mu\text{A}/\text{cm}^2$ ).

The cells were also challenged with various secretagogues. The cells were challenged with apical additions of amiloride ( $10^{-5}$  M), followed by basolateral challenges with isoproterenol ( $10^{-5}$  M), histamine ( $10^{-5}$  M), and bradykinin ( $10^{-7}$  M). The control tissue was time-matched but received no rhuHGF

treatment. The results are illustrated in Figure 7. Amiloride-sensitive sodium absorption in rhuHGF treated cells was dramatically lower than that of control cells ( $2 \mu\text{A}/\text{cm}^2$  vs.  $12 \mu\text{A}/\text{cm}^2$ ). Treatment with rhuHGF did not significantly alter  $R_{te}$  levels from pre-exposure values or time control tissues.

5 It was further found that rhuHGF was most effective when applied to the serosal side of these tissues. One tissue received  $100 \mu\text{l}$  of culture medium containing rhuHGF on its mucosal side, while a control tissue received  $100 \mu\text{l}$  of medium alone. In the treated tissue, pre-exposure values of  $R_{te}$  and  $I_{eq}$  were  $415 \text{ ohm}/\text{cm}^2$  and  $12.5 \mu\text{A}/\text{cm}^2$ ; post exposure values were  $585 \text{ ohm}/\text{cm}^2$  and  $15.2 \mu\text{A}/\text{cm}^2$ . These values were similar to those observed for the control tissue values.

10 The data thus shows that HGF can diminish the abnormally high  $\text{Na}^+$  absorption characteristic of CF-afflicted airway cells to levels comparable to non-CF cells. Although not fully understood, and not wishing to be bound by any particular theory, it is believed that the action of the HGF may result from a down regulation of the ENaC.

15 Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

What is claimed is:

1. A method of treating cystic fibrosis in a mammal, comprising administering an effective amount of hepatocyte growth factor ("HGF") to a mammal diagnosed as having cystic fibrosis.
2. The method of claim 1 wherein said HGF has at least 80% sequence identity with a native  
5 mammalian HGF.
3. The method of claim 2 wherein said HGF has at least 90% sequence identity with a native mammalian HGF.
4. The method of claim 3 wherein said HGF has at least 95% sequence identity with a native mammalian HGF.
- 10 5. The method of claim 4 wherein said HGF is human HGF.
6. A method of reducing sodium ion absorption in mammalian cells, comprising exposing said cells to an effective amount of HGF.
7. The method of claim 6 wherein said mammalian cells are afflicted with cystic fibrosis.
8. An article of manufacture, comprising:  
15 a container;  
a label on said container; and  
a composition contained within said container;  
wherein the composition includes an active agent that is effective for treating cystic fibrosis, the label on said container indicates that the composition can be used for  
20 treating cystic fibrosis, and the active agent in said composition comprises HGF.
9. The article of manufacture of claim 8 further comprising instructions for administering the HGF to a mammal.
10. A kit, comprising:  
25 a first container, a label on said container, and a composition contained within said container;  
wherein the composition includes an active agent that is effective for treating cystic fibrosis, the label on said container indicates that the composition can be used for treating cystic fibrosis, and the active agent in said composition comprises HGF;  
a second container comprising a pharmaceutically-acceptable carrier; and  
instructions for using the HGF to treat cystic fibrosis.

1 / 9

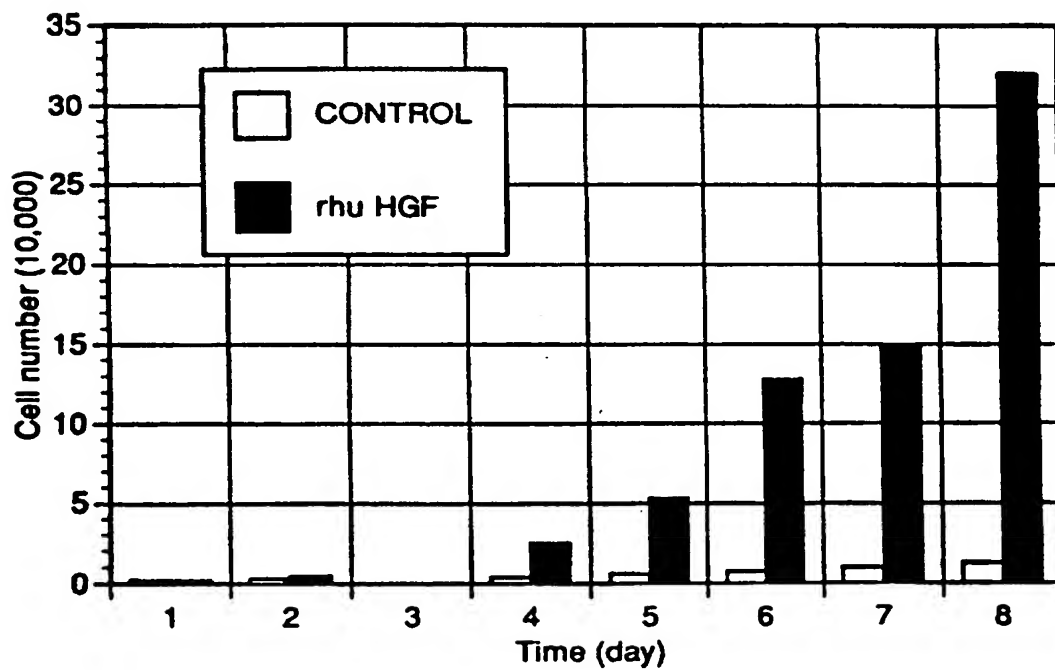


FIG. 1



2 / 9

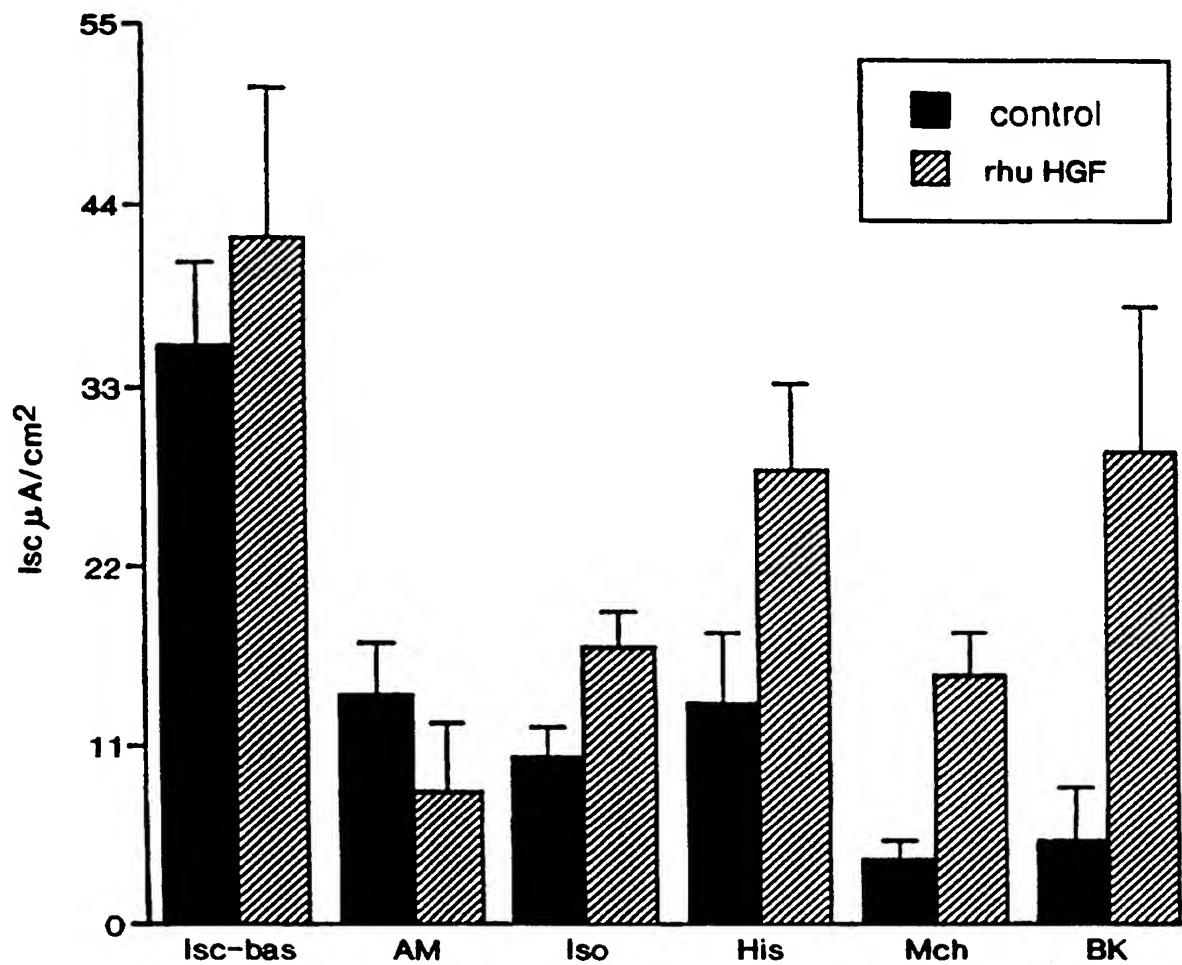


FIG. 2A

3 / 9

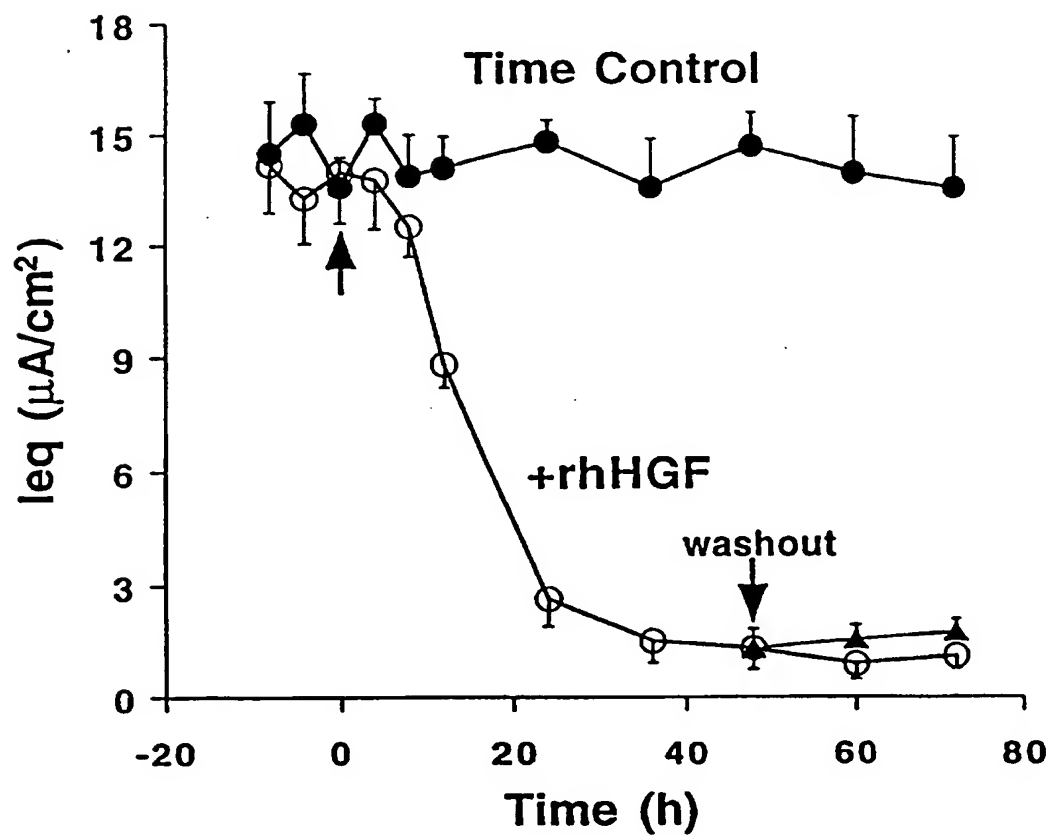


FIG. 2B

4 / 9

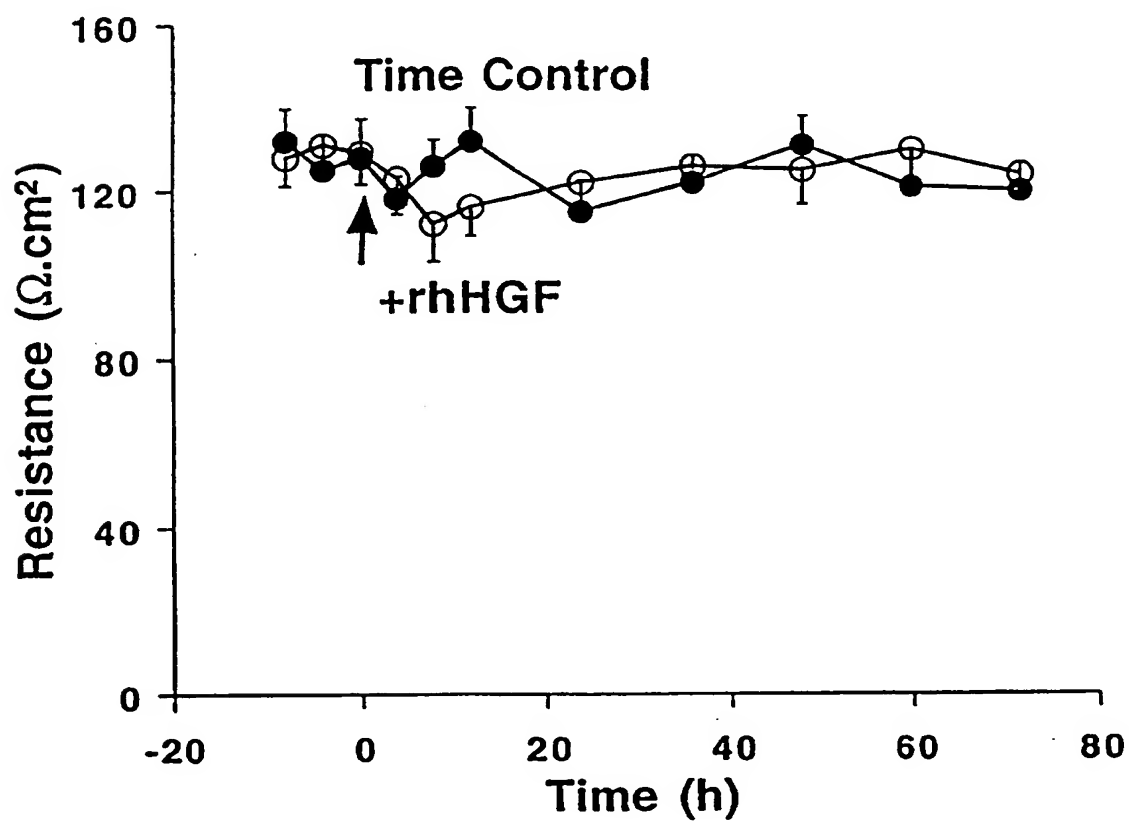


FIG. 2C

5/9

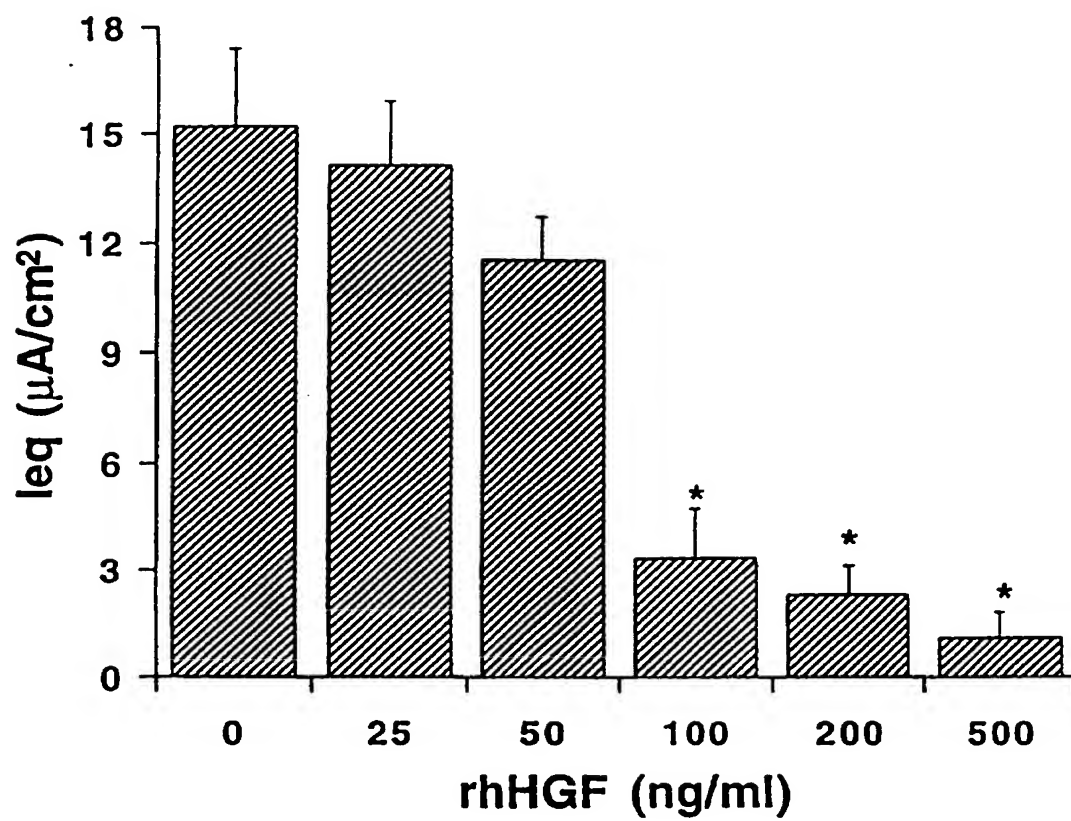
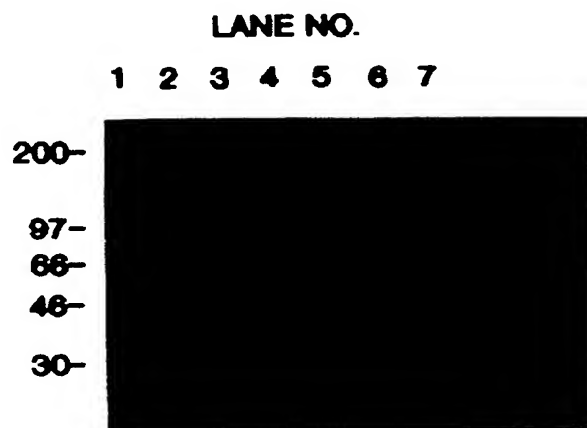
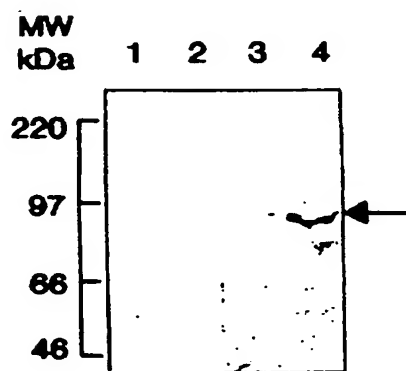


FIG. 2D

6 / 9



**FIG. 3**



**FIG. 4**

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/14588

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF BIOCHEMISTRY, vol. 118, no. 3, September 1995, TOKYO JP, pages 643-649, XP002022365 MATSUDA Y. ET AL.: "Hepatocyte Growth Factor Suppresses the Onset of Liver Cirrhosis and Abrogates Lethal Hepatic Dysfunction in Rats" see the whole document ---	1-10
P,X	WO 95 29694 A (SNOW BRAND MILK PRODUCTS CO.) 9 November 1995 see the whole document --- -/--	1-10



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the international search

9 January 1997

Date of mailing of the international search report

21. 01. 97

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Authorized officer

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/14588

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>CHEST, vol. 109, no. 5, May 1996, page 1413 XP002022363 FUJITA J. ET AL.: "Hepatocyte Growth Factor and Idiopathic Pulmonary Fibrosis" see the whole document -----</p>	1-10

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/14588

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim(s) 1-7  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



### Information on patent family members

PCT/US 96/14588

Form PCT/ISA/210 (patent family annex) (July 1992)